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BCMAp: an integral membrane protein in the Golgi apparatus of human mature B lymphocytes

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Abstract

BCMA is a human gene expressed preferentially in mature B lymphocytes as a 1.2 kb mRNA, which encodes a 184 amino acid peptide (BCMAp). The study of BCMA mRNA expression, using human malignant B cell lines characteristic of different stages of B lymphocyte differentiation, demonstrated that the BCMA mRNA is absent in the pro-B lymphocyte stage. It is expressed faintly at the pre-B cell stage and its expression increases with B lymphocyte maturation. Polyclonal antibodies were used to show, by cellular fractionation and immunoprecipitation, that BCMAp is a non-glycosylated integral membrane protein. Furthermore, BCMAp inserts, *in vitro*, into canine microsomes, as a type I integral membrane protein. Cell surface labeling showed that BCMAp is not expressed in the plasma membrane of mature B lymphocytes. Immunofluorescence studies revealed that BCMAp lies in a cap-like structure near the nucleus, that was identified as the Golgi apparatus by co-localization of BCMAp with CTR433, a marker of the *medial* cisternae of the Golgi apparatus. Confocal scanning laser microscopy of U266 plasma cells labeled with markers of various Golgi apparatus subcompartments strongly suggests that BCMAp is located in the *cis* part of the Golgi apparatus. Thus, BCMAp is the first Golgi resident protein with a tissue specificity and whose expression is linked to the stage of differentiation of B lymphocytes. The location of BCMAp in the Golgi apparatus and its high expression in plasmocytes (secreting large amounts of Ig) suggest that BCMAp is implicated in the intracellular traffic of Ig.

Introduction

Molecular analysis of translocations led to the discovery of new genes which play a role in normal development and differentiation. In a previous paper we reported the molecular analysis of a t(4;16)(q26;p13) translocation, observed in a human T cell lymphoma (1,2). The breakpoints of both chromosome partners involved the IL-2 gene on chromosome 4 and a new gene, termed BCM (referred to as BCMA, for B Cell MAturation, in the current paper), on chromosome band 16p13.1. Transcription resulted in a hybrid IL-2–BCMA mRNA composed of the first three exons of IL-2 fused to the coding sequences of BCMA mRNA. Northern blot and RNase protection assay analyses showed that the BCMA gene is normally expressed as a 1.2 kb transcript in human tissues and cell lines exhibiting B lymphoid features (1,3). These data supported a role for BCMA during B cell development and especially during the final step of B cell maturation. The nucleotide sequence of BCMA cDNA, as well as the deduced protein sequence, have no significant homology with known

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sequences or motifs stored in data banks. Protein algorithm analysis showed that the BCMA peptide contained a central 24 amino acid hydrophobic segment in an α-helix structure, suggesting that BCMAp might be transmembrane, with hydrophilic N- and C-terminals. The deduced protein sequence also indicated the presence of a potential *N*-glycosylation site (Asn42) and several potential phosphorylation sites.

The differentiation of hematopoietic progenitor cells along the B pathway is marked at the molecular level by the synthesis of characteristic cell surface proteins (4), and by the rearrangement of V, D and J elements of Ig genes prior to the expression of Ig heavy chain (HC) and light chain (LC) genes (5). The degree of rearrangement and expression of the Ig is used to characterize each step in differentiation of B cells. Pro-B cells lack both HC and LC proteins, while pre-B cells produce only cytoplasmic μHC (6). Following LC gene rearrangement and expression, the pre-B cells are converted into B cells, which express IgM at their surface. Further maturation leads to Ig-secreting plasma cells, which are the final stage in B cell differentiation. The description of the Ig rearrangement and expression clearly explains the diversity of antibody production, but it is not sufficient to explain the whole process of B cell maturation. The differentiation of B cells is reflected in changes in the morphology of the cells themselves (altered size of the cell, development of the endoplasmic reticulum and of the Golgi apparatus, etc.). Our present state of knowledge does not allow us to say whether or not these cellular changes are directly linked to the phenomenon of Ig rearrangement. The discovery and characterization of new genes that are specifically involved in B cell maturation, like BCMAp, should help to provide a better description and understanding of this process.

This paper describes the biochemical characterization of BCMAp using specific polyclonal antisera. BCMAp was found to be a non-glycosylated membrane-bound polypeptide. Fluorescence microscopy and confocal scanning laser microscopy (CSLM) were used to localize the BCMAp into the Golgi apparatus.

Methods

Cell lines and tissues

Human precursor B cell lines included RS4;11 (7), REH (8), NALM6 (9) and OBS (10,11) cell lines. Human mature B cell lines included the BL31 Burkitt lymphoma B cell line (G. Lenoir, IARC, Lyon, France), the 167 Epstein–Barr virus-transformed B lymphoblastoid cell line (obtained from normal cells in our laboratory), the IgM$^+$ Daudi B cell line (12), the λ$^+$ RPMI 8226 (13) and the IgEλ$^+$ U266 (14) cell lines (American Type Culture Collection, Rockville, MD). Human lymphoid precursor KM3 (15) and human mature T cell MOLT4 (16) cell lines were used as controls. Cells were cultured in RPMI 1640 medium (Gibco/BRL, Paisley, UK) containing 10% FCS (Gibco/BRL).

Preparation of polyclonal anti-BCMA antisera

Polyclonal antiserum to BCMA was obtained by immunizing rabbits with glutathione-S-transferase (GST)–BCMA hybrid proteins expressed in *Escherichia coli* GST fusions were constructed by inserting the 732 bp *Ssp*I–*Eco*RI fragment of BCMA cDNA containing the entire coding sequence, except for a DNA fragment encoding the first 12 N-terminal amino acids, into the plasmid pGEX-2T which had been digested with *Bam*HI, treated with mung bean nuclease to obtain blunt ends and finally digested with *Eco*RI. The correct ligation and in-frame fusion of BCMA to GST in the resulting plasmid pGEX-BCMA4 were tested by sequencing. The GST–BCMA fusion protein was purified from *E. coli* BL21 bacteria containing pGEX-BCMA4 by affinity chromatography on a column of glutathione–Sepharose (Pharmacia, Uppsala, Sweden) (17,18). Peak protein fractions, as judged by A_{280}, were pooled, dialyzed, concentrated under vacuum and stored at –80°C. The purity of the preparations was tested by PAGE. Rabbits were injected subcutaneously with 100 μg protein emulsified in Freund's complete (first injection) and incomplete (subsequent injections) adjuvant. Rabbits were bled and the antisera were tested by ELISA for their capacity to recognize BCMAp.

Affinity purification of anti-BCMA antibodies

Samples of 100 μg of GST and of hybrid GST–BCMA were submitted to SDS–PAGE and transferred to two different nitrocellulose filters. The nitrocellulose band containing the GST protein was used to absorb the anti-GST antibodies present into the anti-BCMA antisera. Anti-BCMA specific antibodies were obtained by affinity-purifying of the pre-absorbed antisera on the nitrocellulose band containing the GST–BCMA protein (19,20). The purified antibodies were concentrated 10-fold, dialyzed against PBS and stored in 50% glycerol at –20°C.

RNase protection assay

Relevant cDNA restriction fragments were subcloned into pGEM-Blue (Promega Biotech, Madison, WI) plasmid vector (3). [α-^{32}P]UTP RNA probes were synthesized from linearized DNA templates by SP6 RNA polymerase (21) using the Riboprobe II system kit (Promega Biotech). Test RNAs (10 μg) were hybridized, at 56°C overnight, with the radiolabeled antisense RNA (3×10^5 c.p.m.) denatured for 5 min at 90°C (22). The samples were thawed on ice, and digested with RNase A (20 mg/ml) and RNase T1 (0.7 U/ml), at 30°C for 45 min (22). The RNase was inactivated with SDS (0.6%) and proteinase K (0.3 mg/ml). The samples were then extracted, ethanol precipitated and analyzed by electrophoresis through a 5% 'Hydrolink long ranger' (J.T. Baker, France) polyacrylamide–7 M urea denaturing gel and autoradiography.

In vitro transcription, translation and translocation

The BCMA cDNA (the entire coding sequence) was cloned under the control of the SP6 polymerase promoter in the *Eco*RI site of the pGEM-4 plasmid. The resulting plasmid (pGEMBCMA5) was linearized with *Hin*dIII and 4 μg of this linearized plasmid was transcribed with SP6 RNA polymerase with an *in vitro* transcription kit. The capped RNA transcripts were purified by phenol–chloroform extraction followed by ethanol precipitation and resuspended in water. Translation in rabbit reticulocyte lysate was performed as suggested by the supplier in a standard volume of 50 μl containing 4 μCi [^{35}S]methionine, in the absence or presence of 2 μl of

canine pancreatic microsomes. The reaction was stopped by incubation with cold methionine and RNase A for 10 min (final concentrations of 10 mM and 0.1 µg/ml respectively). The plasmid and the kits used for the translation experiments were purchased from Promega Biotech. Translation products were denatured by incubation for 4 min at 95°C in sample buffer (25 mM Tris, pH 6.8, 1 mM EDTA, 5% glycerol, 1% β-mercaptoethanol, 4% SDS and 0.005% bromophenol blue) and analyzed by SDS–PAGE on 12.5% polyacrylamide gels (23), which were fixed, treated for fluorography with Entensify (Dupont de Nemours, Brussels, Belgium), dried and exposed on Kodak XAR film at –80°C. When indicated, after translation and translocation microsomal vesicles were separated from the supernatant fraction by centrifugation at 12,000 *g* for 20 min. Aliquots of the resulting supernatant and of the membrane pellet resuspended in water were denatured in sample buffer and directly analyzed by SDS–PAGE, or the membrane pellet was further extracted by carbonate and treated by Endo H.

Carbonate extraction. Since biological membranes are disrupted into open sheets under strong alkaline conditions and are stripped of peripheral proteins (24), carbonate extraction was performed in order to separate peripheral from integral membrane proteins. Aliquots of the membrane pellet were diluted in 0.1 M sodium carbonate, pH 11.5, incubated on ice for 30 min in the presence of protease inhibitors (10 µg/ml leupeptin and pepstatin, 1 µg/ml aprotinin and 1 mM EDTA) and sedimented for 20 min at 2×10^5 *g*. The resulting supernatant was precipitated with 10% TCA. The TCA pellet and the carbonate pellet were suspended in sample buffer and analyzed by SDS–PAGE.

Endoglucosidase treatment. In order to check for protein glycosylation aliquots of the membrane pellet resuspended in water were submitted to 20-fold dilution in 0.1 M sodium citrate, pH 5.5, plus 0.2% SDS and supplemented or not with 1 mU of Endo H (Boehringer, Mannheim, Germany). Incubation was performed for 3 h at 37°C in the presence of protease inhibitors (as indicated above). After addition of a carrier protein, proteins were precipitated with 10% TCA. The TCA pellets were resuspended in sample buffer and analyzed by SDS–PAGE.

Immunoprecipitation of in vitro translated BCMAp

Approximately 1–2 µg of plasmid (pGEMBCMA5) DNA were transcribed using a Promega simultaneous *in vitro* transcription and translation kit, which does not require prior linearization of the plasmid DNA. Translation was performed in a standard 30 µl reaction containing 20 µCi [^{35}S]methionine (Dupont de Nemours), incubated for 1 h at 30°C. The translation mixture was denatured by heating for 5 min at 95°C in sample buffer and further diluted 20-fold with cold TNET buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100), supplemented with 2 mM PMSF, set on ice for 30 min and centrifuged at 15,000 *g* for 20 min at 4°C. The supernatant was cleared by incubating for 1 h at 4°C with pre-immune rabbit serum (10 µl) and Protein A–agarose beads. The resulting supernatant was incubated with agarose Protein A beads and rabbit anti-BCMA unpurified serum (10 µl) for at least 2 h (to overnight) at 4°C and the beads were washed with TNET buffer four times. The immunoprecipitated proteins were extracted with 20 µl 2× sample buffer, heating for 5 min at 95°C and analyzed by SDS–PAGE on a 15% gel (23). The molecular weight markers used were rainbow markers (Amersham, UK). The gel was fixed and treated for fluorography as described above.

In vivo radiolabeling and immunoprecipitation of BCMAp

For *in vivo* radiolabeling experiments, cells were harvested by centrifugation, resuspended in DMEM lacking methionine and cysteine at a concentration of 10^6 cells/ml and incubated for 1 h at 37°C. Cells were labeled by adding 100 µCi/ml Express[^{35}S] (Dupont de Nemours) in the same buffer and incubated at 37°C for 30 min. The labeled cells were harvested, washed three times with PBS (0.15 M NaCl, 0.04 M sodium phosphate, pH 7.4) and then lysed by adding 2% SDS in 50 mM Tris–HCl buffer, pH 6.8 (25 µl/10^6 cells) and heating for 5 min at 95°C. The equivalent of 2×10^6 lysed cells was diluted to 1 ml with cold TNET buffer and proceeded for immunoprecipitation and SDS–PAGE analysis as described above.

Cellular fractionation

Cells were harvested by centrifugation at 800 *g* for 10 min at 4°C, washed with cold PBS and resuspended at 2×10^7 cells/ml. All the experimental manipulations were performed at 4°C. Cells (2×10^7) were disrupted by 20 strokes in a type B Dounce homogenizer. The nuclei were separated by centrifugation at 1000 *g* for 5 min and the supernatant was centrifuged at 10^5 *g* for 30 min in a TL-100 (Beckman, Palo Alto, CA) centrifuge. The resulting supernatant was the cytosolic fraction, while the pellet was the crude membrane fraction. The crude membrane pellet was then treated with 0.2 M sodium carbonate, pH 11.5, incubated for 30 min on ice and centrifuged for 30 min at 10^5 *g*. The resulting new supernatant consisted of peripheral membrane proteins, while the pellet represents the integral membrane proteins.

N-glycanase treatment. Aliquots of the membrane pellet (equivalent of 2×10^6 cells) resuspended in 50 mM Tris, pH 7.6, 2% SDS, were diluted four times with appropriate buffer to a final concentration of 50 mM Tris, pH 7.6, 50 mM β-mercaptoethanol, 50 mM EDTA, 0.5% SDS, denatured by boiling for 5 min and chilled. Nonidet P-40 was added to a final concentration of 7.5%, followed by 0.3 units of *N*-glycanase (Genzyme, Cambridge, MA) plus 2 mM PMSF, and the sample was incubated overnight at 37°C. A control experiment without enzyme was run in parallel. BSA was added as carrier (final concentration: 0.1%) and proteins were precipitated with 10% TCA. The TCA pellets were resuspended in 50 µl of 50 mM Tris, pH 6.8, 2% SDS by heating for 5 min at 95°C, then immunoprecipitated and electrophoresed as described above.

HLA class I molecule and Ig λ chain immunoprecipitation. The supernatant (IgG2a) of the mouse hybridoma W6/32 cell line (25,26) was used to immunoprecipitate the HLA class I molecule, present on U266 cells. Human Ig λ chains, pro-

duced by the U266 cells, were immunoprecipitated using a mouse monoclonal (IgG2aκ) anti-human λ chain antibody (Pierce, Rockford, IL).

Cell surface biotinylation and Western blot analysis

Cells were surface labeled using biotin. After washes in cold PBS, 5×10^7 cells were labeled in 1 ml cold PBS containing 5 mM NHS-LC–biotin [sulfosuccinimidyl-6-(biotinamido) Hexanoate; Pierce] for 1 h at 4°C. After washing once with PBS containing 1 mM glycine and then twice in PBS, the cells were lysed in lysis buffer. Cell lysates were precleared with Protein A–Sepharose and subjected to immunoprecipitation with the respective antibodies. After immunoprecipitation and SDS–PAGE, biotinylated proteins were transferred onto nitrocellulose membrane. The membrane was blocked with 5% BSA in PBS containing 0.1% Tween 20. Proteins were probed for 1 h at room temperature with streptavidin-biotinylated horseradish peroxidase complex (Amersham, UK) diluted 1/3000 in PBS containing 1% BSA, 0.1% Tween 20. For detection, the ECL detection system (Amersham) was used.

Immunocytochemistry procedure

Cells were harvested by centrifugation, washed with PBS and then placed on (500 cells/mm^2) poly-L-lysine (Sigma) coated coverslips for 1 h. Bound cells were fixed with 4% PFA in PBS, pH 7.4, for 10 min, quenched with 20 mM glycine in PBS, washed three times with PBS and permeabilized for 30 min with 0.1% saponin (Sigma) in PBS containing 0.1% BSA (Boehringer Mannheim). The cells were then incubated for 3 h with appropriate primary antibodies in PBS containing 0.1% saponin and 0.1% BSA, washed three times in the same buffer, and incubated for 30 min with the appropriate labeled anti-Ig antibodies diluted in the same buffer. Finally, the cells were rinsed three times with PBS and mounted in a glycerol/PBS solution (Citifluor, London, UK) containing, when necessary, 3 µg/ml DAPI (a dye that stains nuclei) (27). Brefeldin A (2 µg/ml; Sigma) and/or Nocodazole (10 µM; Sigma) were added to the experiments involving disruption of the Golgi apparatus.

The following primary antibodies were used: affinity purified rabbit anti-BCMAp, raised against purified recombinant BCMAp (dilution: 1/10); mouse monoclonal (mAb/36/118, IgG3) anti-human 1,4 β-galactosyltransferase (GalT), a *trans*-Golgi marker (28) (undiluted); mouse mAb (IgG2a), CTR433, a marker of the cisternae of the medial compartment of the Golgi apparatus (29), (1/10); mouse monoclonal (G1/93, IgG1) anti-human ERGIC-53, a protein present in the intermediate compartment (30,31), (undiluted). Primary antibodies were revealed using the following secondary antibodies: sheep fluorescein-conjugated anti-mouse Ig polyclonal antibody (Amersham); donkey Texas Red-conjugated anti-rabbit Ig polyclonal antibody (Amersham). The fluorochrome-conjugated antibodies were used at a dilution of 1/100. A Leitz Aristoplan microscope equipped with epifluorescence optics was used to examine the multilabeled samples at ×100 magnification (numerical aperture 1.32–0.6). Photographs were taken on Kodak Ektachrome (400 ASA) film.

Confocal microscopy analysis

Observations were made with an MRC-1000 (Bio-Rad, Hercules, CA) confocal scanning laser microscope using the Comos software package (Bio-Rad). Discrete photon counting (32) provided a sharp picture of weak label, even with the highest magnification (yielding 29.5 pixels/µm) in (x, y). A multiple-line argon-ion laser beam was operated at full power (25 mW) and attenuated with 1.0, 2.0 or 3.0 neutral density filters. The CSLM system was operated in fluorescence mode for double-fluorescence emission with excitation at 514 nm and a filter separator for fluorescein and rhodamine emissions; two photomultipliers were used in parallel. Simultaneous laser confocal imaging with double fluorescence emission for multiple labeling experiments is often hampered by contamination effects (due to the overlap between fluorescein and rhodamine emission spectra), which were corrected by image processing (33,34). The detection pinhole was closed to a minimum to give the narrowest possible optical section. Other experimental conditions included a 1.40 numerical aperture lens (Nikon Apochromat, Tokyo, Japan) and 1.522 refraction index immersion oil. A distance of 0.4 µm between confocal planes while (z) stepping was chosen to analyze the spatial distribution of the markers throughout the Golgi apparatus. The gain and black level of the PMT were adjusted to optimize the images. Raw confocal data was used to take photographs with a Digital Palette Polaroid CI 3000 camera on Kodak Elite100 films.

Results

Expression of BCMA mRNA in human malignant B cell lines

BCMA mRNA is characteristic of the final step of B cell differentiation (1,3). We attempted to define the mature B cell lines with the highest content of BCMA mRNA to facilitate the study. The RNase protection assay was used to measure the BCMA mRNA for two reasons. First, as there is a natural antisense BCMA RNA in B cell lines (3), we are obliged to use a method of screening that discriminated between the two complementary RNA species, the BCMA and the antisense BCMA; and second, the small amount of BCMA mRNA requires a sensitive method like the RNase protection assay, in which the signal obtained is proportional to the amount of specific RNA present. For this purpose a *Hin*cII BCMA cDNA restriction fragment containing the major part of BCMA coding region was subcloned in a *Sma*I digested pGEM-Blue plasmid vector. This construction was then linearized by *Eco*RI and transcribed *in vitro* by bacteriophage SP6 polymerase, resulting in a 401 nucleotide long transcript. Protection of this transcript by BCMA mRNA followed by RNase treatment gives rise to a 341 nucleotide RNA fragment.

The results obtained using 10 µg of total RNA from several human cell lines are shown in Fig. 1. There is no BCMA mRNA in the control mature T cell line MOLT4. MOLT4 T cells were therefore used as negative controls for BCMA protein throughout this study. The lymphoid precursor cell line KM3 and the pro-B cell line RS4;11 also gave no signal. However, there were faint positive signals in all three pre-B cell lines tested, NALM6, JEA and OB5, indicating that the BCMA

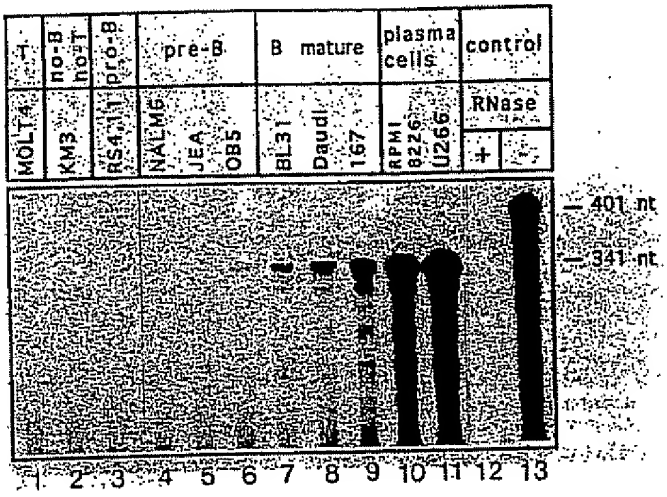

Fig. 1. RNase protection analysis of BCMA gene transcription. Total RNA (10 µg) was used for each assay. RNA was extracted from the following cell lines: MOLT4 (mature T) (lane 1); precursor lymphoid (non-B, non-T) KM3 (lane 2); pro-B RS4,11 (lane 3); pre-B, NALM6 (lane 4), JEA (lane 5) and OB5 (lane 6); mature B, BL31 (lane 7), Daudi (lane 8) and 167 (lane 9); RPMI 8226 (lane 10) and U266 (lane 11) plasma cell lines. Yeast tRNA (20 µg; lane 12) was used as negative control, while 8000 c.p.m. of the 401 nucleotide long undigested probe was present in lane 13.

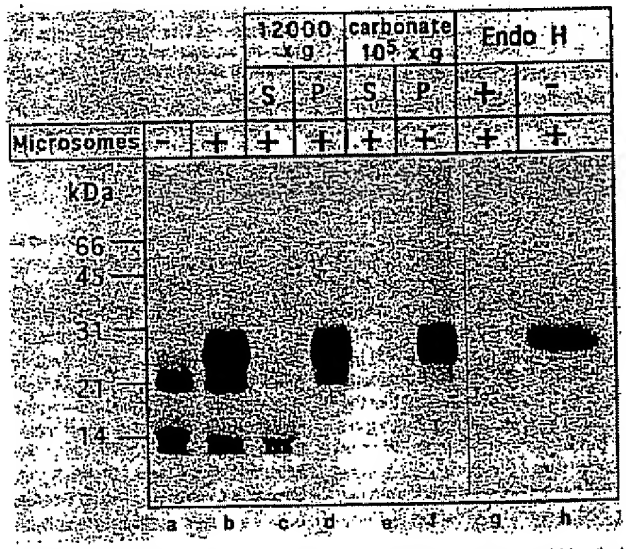

Fig. 2. pGEM4BCMA vector (1 µg) was transcribed *in vitro* and subsequently translated *in vitro* with a rabbit reticulocyte lysate in the absence (lane a) or in the presence (lane b) of canine pancreatic microsomes. The fraction translated in the presence of microsomes was further separated by ultracentrifugation in a soluble supernatant (c) and the crude membrane pellet (d). The crude membrane pellet was treated by alkali and ultracentrifuged into peripheral protein supernatant (e) and integral protein pellet (f). The integral protein fraction was solubilized and treated with Endo H (g); a mock experiment without Endo H was also performed (h).

mRNA is expressed in these lines. There is a greater mRNA expression in the three mature B cell lines, BL31, Daudi Burkitt lymphoma and the 167 lymphoblastoid cell line, but even more expression of BCMA mRNA was obtained with RNA from RPMI 8226 and U266 myeloma (plasma cell) cells. The U266 cell line, which had the highest expression of all

the cell lines tested, was therefore used to characterize and locate BCMAp. These results confirmed previous ones, showing the absence of expression of BCMA mRNA in the stages of B cell differentiation preceding the pre-B step.

BCMAp inserts in vitro into microsomes as a transmembrane glycoprotein

Hydropathy analysis of the deduced sequence of the BCMAp indicates that it lacks an N-terminal signal sequence or a mitochondrial presequence. There is a single apolar predicted α-helical segment of 24 residues further inside the polypeptide (from residue 53 to 76) that could be a potential transmembrane segment. BCMAp carries one potential *N*-glycosylation site in the hydrophilic N-terminus of the protein at residue Asn42. Such a protein is likely to be membrane bound. Membrane-bound polypeptides destined to the endoplasmic reticulum (ER), the Golgi compartments, the plasma membrane, endosomes or lysosomes are initially inserted into the ER membrane before they are sorted to their final destinations. The initial stages of glycosylation of secretory and membrane proteins, when it takes place, occurs during their insertion into the ER. Core oligosaccharides (containing 3Glc, 9Man and *N*-acetylglucosamine) are transferred from a lipid donor to acceptor asparagine residues on the nascent polypeptide chain just as it appears on the lumenal side of the ER membrane (35,36). Isolated rough microsomes have been shown to be able of faithfully reproducing this first step of the secretory pathway; they can promote processing, translocation and glycosylation of a number of secretory and membrane proteins *in vitro*.

To demonstrate that the hydrophobic internal sequence of BCMA could act as a signal anchor sequence, targeting this protein to the ER *in vitro*, we proceeded to transcription and translation of BCMA mRNA in the presence of canine pancreatic microsomes. A pGEM4 vector carrying BCMA cDNA was transcribed and translated *in vitro* with a rabbit reticulocyte lysate system, in the presence of [^{35}S]labeled methionine. The translated product was mainly a 21 kDa polypeptide (Fig. 2, lane a), whose molecular weight is similar to that predicted for the BCMA protein (20,124 Da). The addition of microsomes promoted a shift of BCMAp *in vitro* product from 21 kDa (Fig. 2, lane a) to 23 kDa (Fig. 2, lane b). This band of lower mobility fractionated entirely with the 12,000 *g* pellet (Fig. 2, lane d), while no material was found in the 12,000 *g* supernatant (Fig. 2, lane c) indicating that the 23 kDa species was associated with the microsomes. Upon alkaline extraction all the 23 kDa peptide was recovered in the membrane pellet (Fig. 2, lane f) showing that it was entirely integrated into the ER membrane. To check for protein glycosylation the 12,000 *g* pellet was further treated by Endo H. After incubation in the presence of Endo H, the 23 kDa species shifted back to 21 kDa (Fig. 2, lane g), while the control incubation did not change the electrophoretic mobility (Fig. 2, lane h). Thus, BCMAp is glycosylated *in vitro* in the presence of canine microsomes.

These data indicated that the hydrophobic internal sequence of BCMAp acts as a signal anchor sequence, enabling the targeting and the insertion of this protein to the ER *in vitro*. Furthermore, since the single potential *N*-glycosylation site is in the N-terminal part of the molecule, BCMAp appears as a type I membrane protein (lumenal N-terminal).

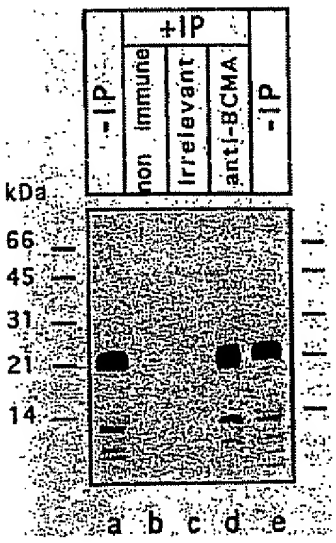

Fig. 3. pGEM4BCMA (1 μg) vector was transcribed *in vitro* and subsequently translated *in vitro* with a rabbit reticulocyte lysate. The translation products were heat-denatured. Immunoprecipitation assays were performed using a non-immune normal rabbit serum (b), an irrelevant polyclonal rabbit antiserum (c), or a specific anti-BCMA polyclonal rabbit antiserum (d). The eluted immunoprecipitated material was analyzed by SDS-PAGE on a 15% gel. Total *in vitro* translated BCMA products without immunoprecipitation were also run (a and e).

Expression of BCMAp in vivo as a non-glycosylated integral membrane protein in mature B lymphocytes (U266 cells)

Polyclonal antibodies to BCMAp fused to GST were raised in rabbits. The *in vitro* translated BCMAp was used to assess the specificity of these polyclonal antibodies for BCMAp. Neither the pre-immune rabbit serum (Fig. 3b) nor an irrelevant rabbit antiserum (Fig. 3c) precipitated any protein, but a 21 kDa peptide was immunoprecipitated with the anti-BCMA antiserum (Fig. 3d). The lower molecular weight peptides in the translation mixture (Fig. 3a and e) and in the specifically immunoprecipitated products (Fig. 3d) were probably due to abnormal translation arrests. Thus the polyclonal rabbit anti-BCMA antiserum immunoprecipitated the BCMAp produced *in vitro*.

U266 B cells and control MOLT4 T cells were labeled and solubilized under denaturing conditions. Proteins were immunoprecipitated with rabbit polyclonal anti-BCMA antiserum. The results (Fig. 4) clearly demonstrate that a 21 kDa peptide was immunoprecipitated from the U266 myeloma cell line (Fig. 4b), which contains large amounts of BCMA mRNA, but not from the control MOLT4 T cell line (Fig. 4a), which contains no BCMA mRNA. These data demonstrate that the BCMAp is expressed *in vivo* by U266 cells.

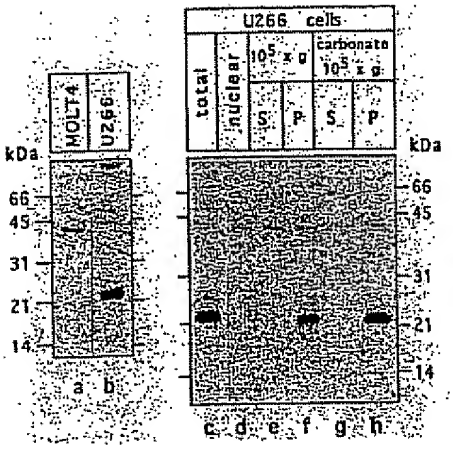

Fig. 4. Subcellular localization of BCMA protein. Cells were labeled with [^{35}S]methionine for 1 h, washed and the equivalent of 2×10^6 cells were denatured by heating at 95°C for 5 min in 50 µl 50 mM Tris, pH 6.8, 2% SDS. Immunoprecipitation was performed as described in Fig. 2. The results obtained using MOLT4 (a) and U266 (b) cells are presented. Cellular fractionation of U266 cells. U266 cell proteins were ^{35}S-labeled for 1 h, washed with cold PBS and disrupted in a Dounce homogeneizer. The nuclear (d) pellet was obtained by centrifugation at 1000 *g*. The postnuclear supernatant was separated into crude cytoplasmic supernatant (e) and crude membrane pellet (f) by ultracentrifugation at 10^5 *g* for 1 h. The crude membrane pellet was further treated with 0.2 M sodium carbonate, pH 11 and separated by ultracentrifugation at 10^5 *g* for 1 h into peripheral membrane protein supernatant (g) and integral membrane protein pellet (h). All these fractions including a total protein fraction (c) were dissolved, immunoprecipitated and electrophoresed as described. Each lane contained the equivalent of proteins prepared from 2×10^6 cells.

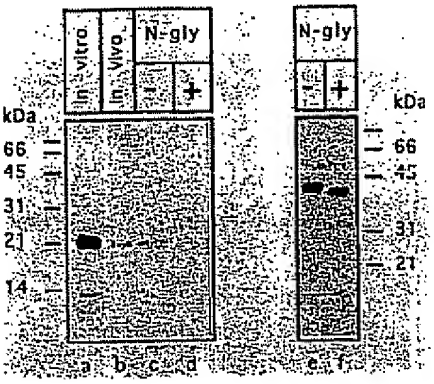

Fig. 5. ^{35}S-labeled crude membrane protein fractions of U266 cells (equivalent of 2×10^6 cells) were used for deglycosylation experiments as described in Methods). The samples after deglycosylation were TCA-precipitated, resuspended in sample buffer, immunoprecipitated and electrophoresed as described in Fig. 3. The mobility of *in vitro* translated BCMAp (a) was compared to that of BCMAp in the untreated crude membrane fraction (b), of mock treated (–*N*-glycanase) (c) and of *N*-glycanase treated sample (d). *N*-glycanase activity was checked by immunoprecipitating, with W6/32 anti-HLA class I antiserum, *N*-glycanase treated (f) and untreated (e) crude membrane samples.

Cell fractionation experiments were performed to test the membrane distribution of BCMAp *in vivo*. U266 cells (^{35}S-labeled) were mechanically disrupted and fractionated. Proteins in the fractions collected were immunoprecipitated with anti-BCMA antiserum and analyzed by SDS–PAGE (Fig. 4). The 21 kDa peptide in the total extract (Fig. 4c) was absent from the nuclear pellet (Fig. 4d), the crude cytosolic supernatant (Fig. 4e) and the peripheral membrane-bound protein supernatant (Fig. 4g). The BCMA polypeptide band was present in the crude membrane (Fig. 4f) and the integral membrane protein (Fig. 4h) pellets. Hence, the BCMA gene is expressed in U266 cells and gives rise to an integral membrane protein *in vivo*.

We determined whether or not BCMAp was *N*-glycosylated *in vivo* by preparing a crude membrane fraction of ^{35}S-labeled U266 B cells and digesting it with the deglycosylating enzyme, *N*-glycanase. The samples were immunoprecipitated with anti-BCMA antiserum and analyzed by SDS–PAGE (Fig. 5). The molecular weight of the immunoprecipitated untreated BCMAp (Fig. 5b) was not altered by *N*-glycanase treatment (Fig. 5d), suggesting that BCMAp is not *N*-glycosylated *in vivo*. This was supported by the fact that the *in vitro* produced unglycosylated BCMA peptide (Fig. 5a) has the same molecu-

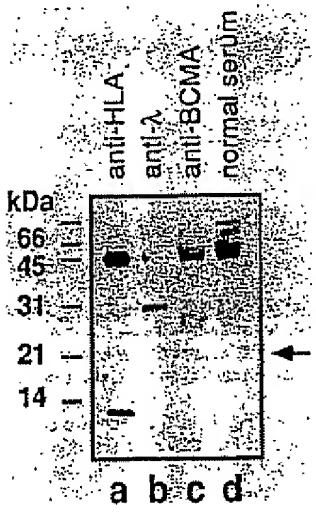

Fig. 6. U266 cells were surface-labeled with NHS-LC-biotin. Detergent extracts of the cells were divided in four fractions and each fraction was immunoprecipitated. (a) Anti-human HLA class I mouse (W6/32) monoclonal antiserum; (b) anti-human Ig λ light chain mouse monoclonal antiserum; (c) anti-BCMA rabbit polyclonal antiserum; (d) normal rabbit serum. Proteins were electrophoresed on 15% polyacrylamide gel, transferred onto nitrocellulose filter and visualized using an ECL detection kit. M, markers are indicated on the left side of the figure. The arrow at the right side of the figure indicates the relative position of BCMAp.

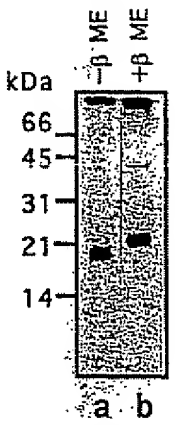

Fig. 7. U266 cells were labeled with [^{35}S]methionine for 1 h, washed and the equivalent of 2×10^6 cells was denatured by 5 min heating at 95°C in 50 µl of 50 mM Tris, pH 6.8, 2% SDS. Immunoprecipitation was performed as described in Fig. 3. The samples were eluted under non-reducing (a) and reducing (b) conditions and electrophoresed as previously described.

lar weight as the *in vivo* expressed BCMAp (Fig. 5b–d). Since these results were in contradiction with the *in vitro* obtained data, the activity of the *N*-glycanase was checked by immunoprecipitating the HLA class I molecules which are normally present in the plasma membrane of U266 cells. *N*-glycanase-treated crude membrane fraction (Fig. 5f) contained a 40 kDa HLA class I peptide of lower molecular weight compared with that (43 kDa) of untreated fraction (Fig. 5e). Difference in glycosylation fate of a protein between results obtained by cell-free translation and *in vivo* expression has already been reported (37) and will be discussed later.

To investigate whether the integral membrane protein BCMAp is expressed or not on the cell surface, we labeled the U266 cell surface proteins using NHS-LC-biotin. Cell lysates were collected and subjected to immunoprecipitation with different antibodies. Figure 6 shows that the W6/32 (anti-HLA class I) and the mouse anti-human λ Ig chain antibodies used as controls precipitate the HLA dimer (Fig. 6, lane a) and the λ chain (Fig. 6, lane b) respectively. Both structures are known to be expressed on the surface of U266 cells. No BCMAp is detected by adding specific polyclonal rabbit anti-BCMA antiserum (Fig. 6, lane c). The two bands of 45 and 27 kDa present in Fig. 6(lane c) are due to non-specific reaction with the excess of rabbit Ig, as it is shown in Fig. 6(lane d), where immunoprecipitation occurred in the presence of normal non-immune rabbit antiserum. These data indicate that BCMAp is not expressed on the surface of the U266 cells.

BCMAp does not form covalent hetero- and/or homopolymers

The deduced amino acid composition of BCMA peptide contains 12 cysteine residues, which could form covalent hetero- and/or homopolymers. The results shown in Fig. 4 indicate that the BCMA peptide is not linked covalently by disulfide bonds to any other polypeptide (heteropolymer). It could, however, form homopolymers. We therefore immunoprecipitated BCMAp, eluted it and subjected it to electrophoresis under reducing (control) and non-reducing conditions (Fig. 7). The BCMA peptide separated under non-reducing conditions (Fig. 7a) has an apparent molecular weight slightly lower than that obtained under reducing conditions (Fig. 7b), indicating that BCMAp does not form covalent hetero- or homopolymers. The same results were obtained (data not shown) even after treatment with 10 mM iodoacetamide, which irreversibly blocks free thiol groups. This observation indicated that cysteines in BCMAp form intra-chain disulphide bonds.

Immunofluorescence studies on the location of BCMAp

The biochemical data obtained validated the predictions of algorithm analysis that the BCMAp was an integral transmembrane protein. We next examined the cellular localization of BCMAp with immunofluorescence studies. Affinity purified anti-BCMAp antibodies do not stain the surface of U266 cells, confirming the results obtained by chemical labeling of the cell surface proteins. Affinity purified anti-BCMAp antibodies were used to stain formaldehyde treated U266 cells permeabilized by saponin. The DAPI dye stained a large nucleus (Fig. 8a), while anti-BCMA antibodies recognized a round shaped structure (Fig. 8b) close to the nucleus (Fig. 8c). The shape and the vicinity to the nucleus suggested that the BCMAp was in the Golgi apparatus. The location of BCMAp in the Golgi apparatus was indeed demonstrated by showing that the structure labeled by anti-BCMA antibodies co-localized with a Golgi marker, a protein of the medial compartment, recognized by the monoclonal CTR433 mouse antiserum (29). Rabbit anti-BCMA antibodies (Fig. 9b) stained the same morphological structure as did the mouse monoclonal CTR433 (Fig. 9a). The two stainings overlapped; when a double discrimination filter was used (data not shown), indicating that the BCMAp lies on the Golgi apparatus.

The fate of BCMA protein in U266 cells treated with Brefeldin A and/or Nocodazole was followed to confirm the localization of BCMAp in the Golgi apparatus. Brefeldin A-treated cells rapidly lose their Golgi as a distinct organelle, and the Golgi contents and membranes are redistributed in the ER (38). This effect is reversible, because removal of Brefeldin A results in the rapid sorting of Golgi components out of the ER and reformation of the Golgi apparatus (31). U266 cells were treated with Brefeldin A for 5, 10, 15, 30 or 90 min at 37°C, fixed, permeabilized, and stained for BCMA and CTR433 antigen using indirect immunofluorescence. To show the reversibility of Brefeldin A action, U266 cells treated with Brefeldin A for 90 min were incubated without Brefeldin A for a further 90 min at 37°C, fixed and stained. The distributions of BCMAp (Fig. 10, right panels) and CTR433 antigen (Fig. 10, left panels) were strikingly changed by incubation for 5 min with Brefeldin A. Incubation with Brefeldin A for 15 min caused BCMAp and CTR433 to be completely redistributed in the cytoplasm. The two staining patterns were almost identical, with some small differences after dispersal of the Golgi apparatus within the cytoplasm following redistribution in the ER. Removal of Brefeldin A resulted in the reformation

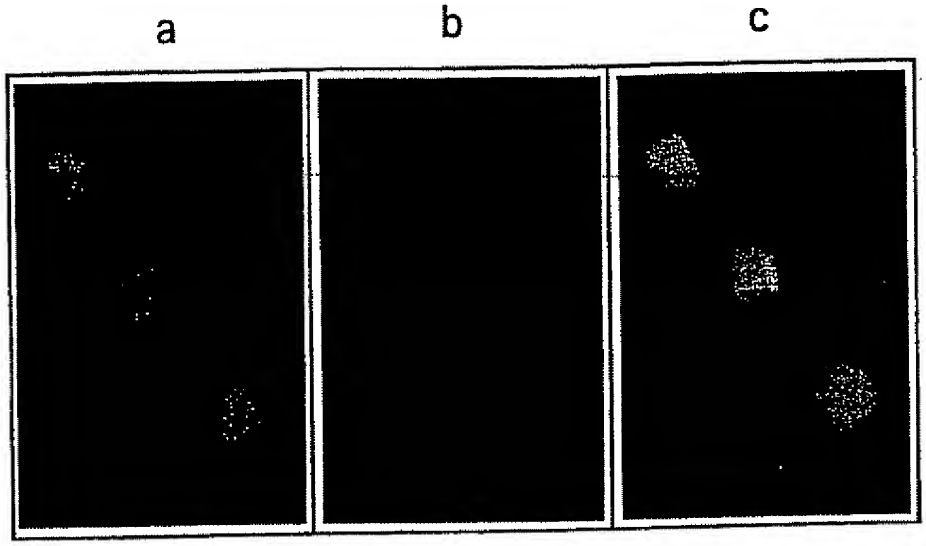

Fig. 8. Immunocytochemical labeling of U266 cells with anti-BCMA antiserum. U266 cells were fixed with PFA, stained with anti-BCMA antiserum, and visualized with a secondary Texas-Red-labeled anti-rabbit antiserum. The preparation was mounted in the presence of DAPI, a nuclear stain. Color photographs were taken with a DAPI-specific filter (a), a Texas Red filter (b) and a double exposure with both the two filters (c).

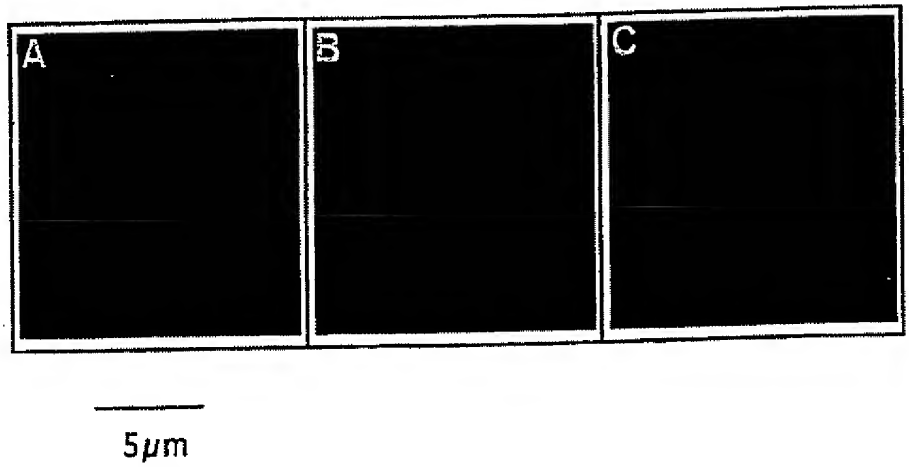

Fig. 12. U266 cells were fixed with 4% formaldehyde, double-labeled with appropriate antibodies and analyzed by CSLM. Sequential micrographs were taken at 400 nm intervals along the z-axis at excitation wavelengths of 488 nm for FITC, 550 nm for Texas Red and 357 nm for DAPI (barrier filter 400 nm). The nucleus of the cells (in A and B) has been stained by incorporating DAPI in the mounting solution. One section for each double-labeling experiment is shown. The results are shown in false colors. (A) ERGIC-53, IC marker (green), BCMAp (red) and DAPI (blue) (B) CTR433, *medial* Golgi compartment marker (green), BCMAp (red) and DAPI (blue). (C) GalT, *trans* Golgi compartment marker (red) and BCMAp (green).

of the Golgi apparatus (Fig. 10, bottom). There were also some slight differences between the two staining patterns

Nocodazole disrupts the microtubule structure of cells by inhibiting polymerization of tubulin monomers (39). This results in fragmentation of the Golgi apparatus into numerous structures of varying sizes that are distributed throughout the

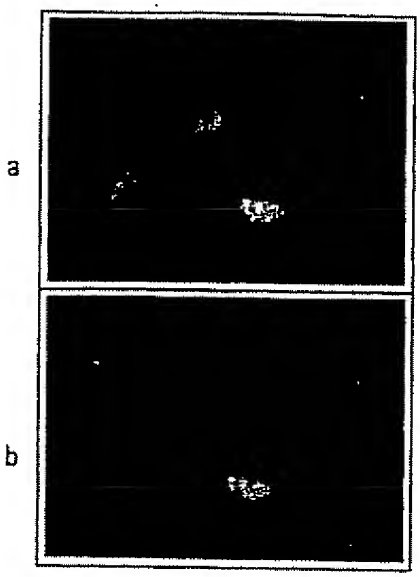

Fig. 9. U266 cells were double-stained with the mouse monoclonal CTR433 antiserum, a marker of the Golgi apparatus and the anti-BCMAp specific rabbit polyclonal antiserum. A FITC-conjugated anti-mouse IgG antiserum was used to reveal CTR433 (a), while a Texas-Red-conjugated anti-rabbit IgG antiserum was used to reveal BCMAp (b).

cytoplasm. U266 cells were treated with Nocodazole for 5, 15, 30 and 60 min, fixed, and stained for BCMAp and CTR433 antigen. Disruption of the Golgi apparatus had begun by 5 min (Fig. 11) and continued to increase up to 1 h, at which time the Golgi apparatus was totally disrupted. The CTR433 (Fig. 11, left panel) and BCMAp (Fig. 11, right panel) stainings were almost identical, but with subtle differences in the distribution of the two markers. These differences continued even after 60 min of Nocodazole treatment. Thus the immunofluorescence data show that BCMAp is located in the Golgi apparatus, but that BCMAp and CTR433 may be in different subcompartments of the Golgi apparatus.

Confocal microscopy: BCMAp lies neither in the 'intermediate compartment' (IC) nor in the trans compartment of the Golgi apparatus

The Golgi apparatus is composed of a set of morphologically and functionally discrete compartments and it is organized into three functionally distinct regions, the *cis*-Golgi network (CGN), which has also been named the 'salvage compartment' and the IC, the Golgi stack (containing topologically separate compartments, which seem to be three, i.e. *cis*, *medial* and *trans*), and the *trans*-Golgi network (TGN) (40–42). Immunocytochemical labeling followed by electron microscopy studies has always been used to localize precisely a protein in one of the different compartments of the Golgi apparatus.

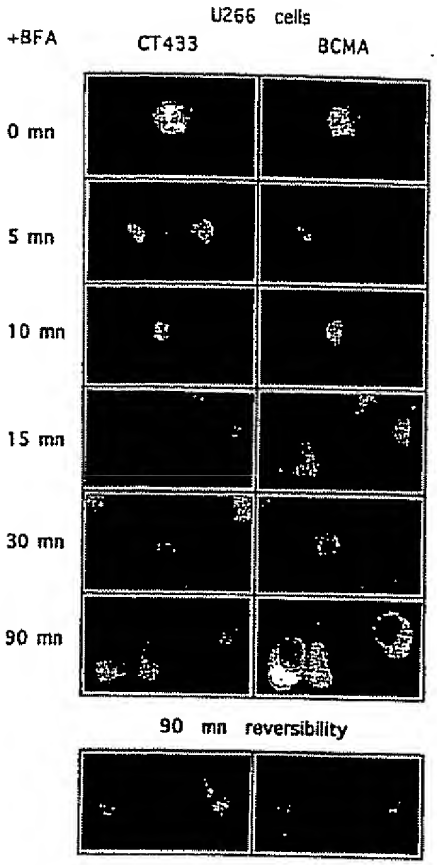

Fig. 10. Disruption of the Golgi apparatus of U266 cells with Brefeldin A. U266 cells were harvested, incubated for various times with 2 µg/ml of Brefeldin A and fixed with PFA. After 90 min of Brefeldin A treatment the cells were washed and resuspended in RPMI, supplemented with 10% FCS, incubated for 90 min at 37°C to check the reversibility of the Brefeldin A action and fixed with PFA. They were then double-stained with the CTR433 monoclonal and the anti-BCMAp rabbit polyclonal antisera.

Our attempts to use the affinity purified polyclonal anti-BCMA antibodies in electron microscopy studies were unsuccesful. The use of CSLM in double-labeling experiments of Golgi compartments with appropriate markers was an alternative way to answer the question of localization of BCMAp. This technique has already permitted the observation, in the Golgi apparatus, of two distinct or mixed staining patterns according to the relative positions of the selected markers (43). Since only few antibodies raised against Golgi antigens are available and many of them are species-specific, we selected the most convenient markers for double-labeling experiments. We have used three mouse mAb, which are well defined. CTR433

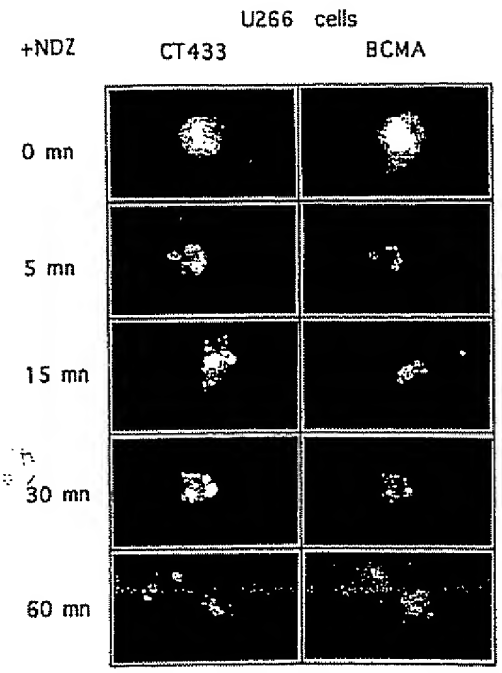

Fig. 11. Disruption of the Golgi apparatus of U266 cells with Nocodazole. U266 cells were incubated with 10 μM Nocodazole, fixed at various times, and double-stained with the CTR433 monoclonal and the anti-BCMAp rabbit polyclonal antisera.

(IgG2a) appears to label the *medial* cisternae, staining an unknown protein (29), the mAb/36/118 (IgG3) anti-GalT antibody stains the *trans* compartment of the Golgi apparatus (28), while the mAb G1/93 (IgG1) antibody stains ERGIC-53, a membrane protein of the ER–Golgi IC (30). Unfortunately we do not dispose of a well-defined mAb staining the human *cis* compartment of the Golgi apparatus. In order to investigate the relative position of BCMAp to the intermediate compartment and *medial* and *trans* compartments of the Golgi apparatus, double-labeling experiments were performed on U266 cells and analyzed with CSLM. Twenty six 400 nm serial sections along the z-axis of a stained U266 cell were analyzed. ERGIC-53 showed a vesicular localization (green) dispersed in the cytoplasm, while BCMAp was present on a continuous structure (red) forming a cap to the nucleus (blue). The two proteins do not mainly co-localize in all sections analyzed, except at a few points (Fig. 12A). Furthermore a partial co-localization (yellow) of BCMAp (red) with the medial compartment of the Golgi apparatus (green), defined by the labeling with the CTR433 antibody, has been observed (Fig. 12B). On the contrary no co-localization of BCMAp (green) and GalT (red) has been found, allowing the conclusion that BCMAp is not located into the *trans* compartment of the Golgi apparatus (Fig. 12C).

Discussion

In previous studies we have characterized the BCMA gene (1,3) as a gene expressed preferentially in mature B cells. In this study we show that BCMA mRNA expression correlates with the final steps of B cell differentiation and that it is not expressed before the pre-B stage. The BCMA gene has no significant homology with known nucleotide and/or protein sequences. As it does not contain any known functional motif, it was difficult to suggest a role the BCMA gene/protein could play in B cell maturation. This work provides further information on the BCMA gene by studying its expression as a protein (BCMAp). The biochemical characterization of the BCMAp and its cellular localization should give some clues as to its function.

Anti-BCMAp polyclonal antibodies used in immunoprecipitation experiments showed that the expression of BCMAp correlates with the expression of BCMA mRNA: BCMAp is produced in differentiated mature B cells, but not in T cells. Cell fractionation and carbonate treatment followed by immunoprecipitation showed that BCMAp is an integral membrane protein, as was suggested by the protein algorithm analysis. It is localized neither in the nucleus, nor in the plasma membrane, but is located in the Golgi apparatus as detailed later on.

The 184 residue BCMAp has a single predicted transmembrane segment (amino acids 53–76) and could be oriented in the Golgi membrane either as a type I (lumenal N-terminal and cytoplasmic C-terminal) or as a type II (lumenal C-terminal and cytoplasmic N-terminal) membrane protein. The transmembrane orientation of integral membrane proteins following the secretory pathway is defined during their insertion into the ER membrane. This process is thought to depend upon the nature of the hydrophilic residues flanking an hydrophobic segment. Combining the so-called 'positive inside rule' (44) and the 'net charge difference' across the transmembrane segment (45) has been proposed to allow prediction of the topology of membrane spanning of eukaryotic proteins (46). The analysis of BCMAp sequence according to these rules and using the TOP-PRED2 program (46) cannot define the transmembrane topology of BCMAp with certainty. However, the highly dissymetric composition in charged residues of BCMAp (30% of residues of the hydrophilic C-terminus are charged, in contrast to only 7% in the hydrophilic N-terminus) would be indicative of a cytoplasmic orientation of the C-terminus of the protein (46,47).

As *N*-glycosylation occurs only in the lumen of the ER (35, 36) and the only potential *N*-glycosylation site of the BCMAp is in the N-terminal part of the molecule, checking whether BCMAp is glycosylated appeared as an easy experimental way to determine its topology. This was tested by translating BCMA mRNA in the presence of canine pancreatic microsomes, which faithfully reproduce the insertion and glycosylation of membrane proteins in their correct orientation. BCMAp was found to be inserted *in vitro* into the microsome membrane as a type I integral glycoprotein, as strongly suggested by the abundance of charged residues in its C-terminus. Thus, *in vivo* expressed BCMAp is likely to be a type I membrane protein. Surprisingly, BCMAp was found unglycosylated

in vivo. Consensus glycosylation sites, even lumenally located, are not always glycosylated. A survey of 115 human single span type I membrane proteins indicated that the closest utilized consensus site is located 16 residues from the membrane domain (37). Experimental data obtained from *in vitro* experiments suggested that a minimum spacing of 14–15 residues is necessary for the utilization of a consensus *N*-glycosylation site, which precedes a transmembrane segment (48). The potential glycosylable Asn residue in BCMAp (residue 42) is located only 10 residues upstream from the predicted transmembrane segment. This distance might be too short to enable glycosylation *in vivo*. *In vitro* glycosylation on lumenal Asn residues that do not undergo *in vivo* glycosylation has been reported in several instances (37). Protein synthesis, folding and glycosylation *in vitro* occur at lower rates than *in vivo*, perhaps allowing secondary glycosylation events (36). It might also be noted that glycosylation occurs preferentially on the consensus site Asn-X-Thr, compared to Asn-X-Ser, as exhibited by BCMAp (36).

Immunofluorescence staining was used to investigate the subcellular localization of BCMAp. The anti-BCMA antisera stained a round complex structure adjacent to the nucleus. BCMAp co-localized with the Golgi marker, stained by the CTR433 antibody (29), indicating that BCMAp lies in the Golgi apparatus. The staining obtained by the anti-BCMAp antisera was dispersed in the ER of the cells by adding Brefeldin A, in the same way as did the staining obtained by CTR433. Similar data were obtained after treatment with Nocodazole. These observations further conforted the Golgi localization of BCMAp. Most of the Golgi proteins located in the compartments of the Golgi stack (*cis*, *medial* and *trans*) redistribute to ER (31,49), after treatment with Brefeldin A, which inhibits the anterograde pathway from ER to the Golgi apparatus (31). The TGN is a Golgi compartment that fails to enter the ER after Brefeldin A treatment (50–52). The localization of the BCMAp in the ER after addition of Brefeldin A suggests that BCMAp is not located in the TGN. Finally p58 (53) and p53 (ERGIC-53) (31), the two proteins characteristic of the IC, do not modify their localization since IC preserves its identity in the presence of Brefeldin A. In addition two recent papers describing p210 (54) and gp74 (55), two proteins located in the CGN (considered by the authors as a Golgi compartment distinct of the IC), showed that p210 and gp74 redistribute in the IC upon Brefeldin A treatment. Thus, it is more likely that BCMAp is located in one of the three parts of the Golgi stack (*cis*, *medial* or *trans*).

As immuno-electron microscopy experiments to localize BCMAp were unsuccesful for the moment, confocal microscopy was used to compare the relative localizations of BCMAp, ERGIC-53 (IC), CTR433 (*medial* cisternae of the Golgi apparatus) and GalT (*trans* compartment of the Golgi apparatus). One obvious limitation for these experiments was the paucity of accepted marker proteins for individual subcompartments, in particular for the entry face of the Golgi stack, the *cis* part. A second limitation of this work is the interpretation of the results obtained by CSLM. A full separation, such as that obtained between BCMAp and GalT (Fig. 12C), can be easily interpreted. BCMAp and GalT do not co-localize. A full separation has also been observed between ERGIC-53 and BCMAp, indicating that they do not co-localize. On the contrary the partial overlap of the patterns obtained by anti-BCMAp and CTR433 antibodies raises the problem of the origin of this overlap. Such an overlap is difficult to analyze even under well-defined oversampling conditions because of the size of the Golgi compartments, which are close to the resolution of the CSLM (43). Finally, the confocal microscopy results indicate that BCMAp is not located in the *trans*, but it may lie either in the *cis* or the *medial* compartments of the Golgi apparatus. These results have to be confirmed by electron microscopy experiments. We are now developing new polyclonal and monoclonal antibodies to be used. In any way, all the Golgi resident proteins, described to date, seem to be expressed in all cells. Our previous results (1,3), which showed a preferential expression of BCMA in B mature cells, indicate that BCMAp is the first Golgi-resident protein with a tissue specificity.

There is strong evidence that the Golgi system plays a pivotal role in the post-translational modification and distribution of proteins co-translationally sorted in the ER (i.e. secretory, plasma membrane, lysosomal, Golgi and viral proteins) or internalized by endocytosis (plasma membrane proteins and ligands, organelle proteins transiting through the plasma membrane) (42,56). These functions are performed by the stack of Golgi cisternae and the TGN. Enzymes that are asymmetrically distributed through the cisternae introduce a variety of post-translational modifications (i.e. glycosylation, sulfation, acylation, proteolytic digestion) in newly-synthesized proteins and proteins internalized by endocytosis. The *cis* side of the Golgi stack receives newly synthesized material from the ER. In addition to serving as a site of entry into the Golgi, recent evidence suggests that the CGN is involved in the recycling of protein and lipid components back to the ER, while having a relatively limited role in glycosylation. The *cis* Golgi stack has no specific role as yet. Mature B lymphocytes have two main functions, the production and secretion of Ig, and a second one, the presentation of antigens to $CD4^+$ T cells via the class II HLA molecules located on B cell surface.

The localization of the BCMA protein in the Golgi apparatus of mature B cells therefore suggests three possible functions for the BCMAp. First, it might play a role in antigen processing and presentation by the HLA class II molecules. Secondly, it could be implicated in the intracellular traffic and/or post-translational modifications of Ig. Finally, it might be linked to an as yet unknown function of mature B cells. The first function of participation in the association of HLA class II molecules with the processed antigen or permitting the flow of the already formed molecules through the Golgi is inconsistent with the fact that there are large amounts of BCMAp in plasma cells, which carry little or no HLA class II molecules (4). The second function, which assumes a specifically expressed protein (BCMAp) only for the Ig post-translational changes, is contradicted by the fact that the Ig do not need any special modification, which distinguishes them from other proteins. However, it is possible that BCMAp could be part of a protein complex which facilitates the secretion of the Ig. This is consistent with the high concentration of BCMAp in plasma cells producing and secreting large amounts of Ig. However, it is always possible that BCMAp participates in another, as yet unknown, function of B lymphocytes. We are now testing the above hypotheses.

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Abbreviations

CGN	*cis*-Golgi network
CSLM	confocal scanning laser microscopy
ER	endoplasmic reticulum
GST	glutathione-S-transferase
IC	intermediate compartment
TGN	*trans*-Golgi network

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